

CLAIMS

1. A method for screening or selecting cells expressing a desired level of a polypeptide, comprising:

5 a) providing a plurality of cells each comprising an expression cassette comprising a first polynucleotide encoding the polypeptide, at least one stop codon downstream of the first polynucleotide, and a second polynucleotide encoding a cell membrane anchoring peptide, a reporter peptide or an epitope tag downstream of the stop codon;

b) cultivating the cells in the presence of a termination suppression agent under
0 conditions that allow expression of the polypeptide; and

c) selecting at least one cell expressing the polypeptide fused to a cell membrane anchoring peptide, a reporter peptide or an epitope tag.

2. A method for evaluating recombinant polypeptide expression in a population of cells,
5 comprising:

a) providing a plurality of cells each comprising an expression cassette comprising a first polynucleotide encoding a recombinant polypeptide, at least one stop codon downstream of the first polynucleotide, and a second polynucleotide encoding a cell
0 membrane anchoring peptide, a reporter peptide or an epitope tag downstream of the stop codon;

b) cultivating the cells in the presence of a termination suppression agent under conditions that allow expression of a fusion protein comprising the recombinant polypeptide and the cell membrane anchoring peptide, reporter peptide or epitope tag; and

c) sorting the cells to select at least one cell expressing the fusion protein at a
5 desired level and/or with a desired uniformity.

3. A method for screening or selecting at least one cell expressing a polypeptide with a desired binding affinity to a ligand from cells expressing a library of polypeptide variants, comprising:

0 a) providing a plurality of cells each comprising an expression cassette comprising a first polynucleotide encoding a polypeptide variant, at least one stop codon downstream of the first polynucleotide, and a second polynucleotide encoding a cell membrane anchoring peptide, a reporter peptide or an epitope tag downstream of the stop codon;

b) cultivating the cells in the presence of a termination suppression agent under conditions that allow expression of the polypeptide variant; and

c) selecting at least one cell expressing the polypeptide variant fused to a cell membrane anchoring peptide based on binding affinity of said polypeptide variant to said
5 ligand.

4. The method of any of the preceding claims, wherein the termination suppression agent is an aminoglycoside antibiotic.

10 5. The method of any of the preceding claims, wherein the cells are screened or selected by FACS.

6. The method of any of the preceding claims, wherein the second polynucleotide encodes a cell membrane anchoring peptide, and wherein the at least one selected cell
15 expresses a fusion protein comprising the polypeptide fused to a cell membrane anchoring peptide, the fusion protein being displayed at the surface of said cell.

7. The method of claim 6, wherein the cell membrane anchoring peptide is a GPI anchor.
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8. The method of any of claims 1-5, wherein the second polynucleotide encodes a reporter peptide or an epitope tag.

9. The method of claim 8, wherein the second polynucleotide encodes a reporter
25 peptide selected from the group consisting of green fluorescent protein (GFP), luciferase, β -galactosidase, β -glucuronidase and chloramphenicol acetyltransferase (CAT).

10. The method of claim 9, wherein the second polynucleotide encodes an epitope tag selected from the group consisting of V5, His, FLAG™, HA, c-Myc, VSV-G, and HSV.
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11. The method of any of claims 8-10, wherein the expression cassette further comprises a polynucleotide encoding a cell membrane anchoring peptide.

12. The method of any of claims 4-11, wherein the aminoglycoside antibiotic is selected from the group consisting of G-418, gentamicin (gentamycin), paromomycin, hygromycin, amikacin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin and tobramycin.

5 13. The method of any of the preceding claims, wherein the cell is a eukaryotic cell.

14. The method of claim 13, wherein the cell is selected from the group consisting of mammalian cells, filamentous fungal cells, yeast cells and insect cells.

10 15. The method of any of the preceding claims, further comprising:

d) cultivating at least one selected cell in the absence of a termination suppression agent to obtain expression of the polypeptide as a soluble polypeptide.

15 16. A method for alternately expressing i) a soluble, untagged polypeptide or ii) a membrane-bound or tagged polypeptide from a single cell or cell line, comprising:

a) providing a plurality of cells each comprising an expression cassette comprising a first polynucleotide encoding the polypeptide, at least one stop codon downstream of the first polynucleotide, and a second polynucleotide encoding a cell membrane anchoring peptide, a reporter peptide or an epitope tag downstream of the stop codon;

20 b) cultivating the cells in the presence of a termination suppression agent under conditions that allow expression of the polypeptide;

c) selecting at least one cell expressing the polypeptide fused to a cell membrane anchoring peptide, a reporter peptide or an epitope tag; and

25 d) cultivating said selected cell in the absence of a termination suppression agent to obtain expression of the polypeptide as a soluble polypeptide.

17. The method of claim 16, wherein the termination suppression agent is an aminoglycoside antibiotic.

30 18. The method of claim 16 or 17, wherein the cells are screened or selected by FACS.

19. The method of any of claims 16-18, wherein the second polynucleotide encodes a cell membrane anchoring peptide, and wherein the at least one selected cell expresses a fusion

protein comprising the polypeptide fused to a cell membrane anchoring peptide, the fusion protein being displayed at the surface of said cell.

20. The method of claim 19, wherein the cell membrane anchoring peptide is a GPI
5 anchor.

21. The method of any of claims 16-18, wherein the second polynucleotide encodes a reporter peptide or an epitope tag.

10 22. The method of claim 21, wherein the second polynucleotide encodes a reporter peptide selected from the group consisting of green fluorescent protein, luciferase, β -galactosidase, β -glucuronidase and chloramphenicol acetyltransferase (CAT).

23. The method of claim 22, wherein the second polynucleotide encodes an epitope tag
15 selected from the group consisting of V5, His, FLAGTM, HA, c-Myc, VSV-G, and HSV.

24. The method of any of claims 21-23, wherein the expression cassette further comprises a polynucleotide encoding a cell membrane anchoring peptide.

20 25. The method of any of claims 17-24, wherein the aminoglycoside antibiotic is selected from the group consisting of G-418, gentamicin (gentamycin), paromomycin, hygromycin, amikacin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin and tobramycin.

25 26. The method of any of claims 16-25, wherein the cell is a eukaryotic cell.

27. The method of claim 26, wherein the cell is selected from the group consisting of mammalian cells, filamentous fungal cells, yeast cells and insect cells.

30 28. A method for alternately expressing i) a membrane-bound, untagged polypeptide or ii) a membrane-bound, tagged polypeptide from a single cell or cell line, comprising:

a) providing a plurality of cells each comprising an expression cassette comprising a first polynucleotide encoding the polypeptide and a cell membrane anchoring peptide, at

least one stop codon downstream of the first polynucleotide, and a second polynucleotide encoding a reporter peptide or an epitope tag downstream of the stop codon;

b) cultivating the cells in the presence of a termination suppression agent under conditions that allow expression of the polypeptide and the cell membrane anchoring peptide;

5 c) selecting at least one cell expressing a fusion protein comprising the polypeptide, the cell membrane anchoring peptide, and a reporter peptide or an epitope tag; and

d) cultivating said selected cell in the absence of a termination suppression agent to obtain expression of a protein comprising the polypeptide in membrane-bound form without
10 the reporter peptide or epitope tag.

29. The method of claim 28, wherein the termination suppression agent is an aminoglycoside antibiotic.

15 30. The method of claim 28 or 29, wherein the cells are screened or selected by FACS.

31. The method of any of claims 28-30, wherein the cell membrane anchoring peptide is a GPI anchor.

20 32. The method of any of claims 29-31, wherein the aminoglycoside antibiotic is selected from the group consisting of G-418, gentamicin (gentamycin), paromomycin, hygromycin, amikacin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin and tobramycin.

25 33. The method of any of claims 28-32, wherein the cell is a eukaryotic cell.

34. The method of claim 33, wherein the cell is selected from the group consisting of mammalian cells, filamentous fungal cells, yeast cells and insect cells.

30 35. The method of any of claims 28-34, wherein the second polynucleotide encodes a reporter peptide selected from the group consisting of green fluorescent protein, luciferase, β -galactosidase, β -glucuronidase and chloramphenicol acetyltransferase (CAT).

36. The method of any of claims 28-34, wherein the second polynucleotide encodes an epitope tag selected from the group consisting of V5, His, FLAG™, HA, c-Myc, VSV-G, and HSV.

5 37. A method for screening or selecting cells expressing a polypeptide of interest from a population of cells, comprising:

a) transfecting a population of cells with an expression cassette comprising, in sequence, a gene of interest, at least one stop codon, and a cell targeting peptide, wherein the expression cassette does not comprise an antibiotic resistance gene;

10 b) cultivating the transfected population of cells in the presence of a termination suppression agent; and

c) selecting at least one cell expressing the polypeptide fused to a cell targeting peptide.

15 38. The method of claim 37, wherein the cell targeting peptide is selected from the group consisting of cell membrane anchoring peptides, nuclear localization signals, signals targeting the polypeptide to a non-nuclear sub-cellular compartment, and cellular structures.

39. The method of claim 38, wherein the cell targeting peptide is selected from the group
20 consisting a GPI anchor; a signal targeting the polypeptide to the cytoplasm, mitochondria or endoplasmic reticulum; and microtubules.

40. The method of any of claims 37-39, wherein the termination suppression agent is an aminoglycoside antibiotic.

25 41. The method of claim 40, wherein the aminoglycoside antibiotic is selected from the group consisting of G-418, gentamicin (gentamycin), paromomycin, hygromycin, amikacin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin and tobramycin.

30 42. The method of any of claims 37-41, wherein the cells are screened or selected by FACS.

43. The method of any of claims 37-42, further comprising:

d) cultivating at least one selected cell in the absence of a termination suppression agent to obtain expression of the polypeptide without the cell targeting peptide.

44. A method for screening or selecting cells expressing a polypeptide of interest from a population of cells, comprising:

a) transfecting a population of cells with an expression cassette comprising, in sequence, a gene of interest, at least one stop codon, and an antibiotic resistance gene, wherein the antibiotic resistance gene provides resistance to a non-aminoglycoside antibiotic;

b) cultivating the transfected population of cells in the presence of an aminoglycoside antibiotic and the non-aminoglycoside antibiotic; and

c) selecting at least one cell which is able to grow in the presence of the non-aminoglycoside antibiotic.

45. The method of claim 44, wherein the aminoglycoside antibiotic is selected from the group consisting of G-418, gentamicin (gentamycin), paromomycin, hygromycin, amikacin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin and tobramycin.

46. The method of claim 44 or 45, wherein the non-aminoglycoside antibiotic is selected from the group consisting of ampicillin, bleomycin, phleomycin, spectinomycin, blasticidin, puromycin and zeocin.

47. The method of any of claims 44-46, wherein the cells are screened or selected by FACS.

48. The method of any of claims 44-47, further comprising:

d) cultivating at least one selected cell in the absence of any antibiotic to obtain expression of the polypeptide without expression of the antibiotic resistance gene.

49. The method of any of the preceding claims, wherein the polypeptide is selected from an antibody or antibody fragment, a plasma protein, an erythrocyte or thrombocyte protein, a cytokine, a growth factor, a profibrinolytic protein, a binding protein, a protease inhibitor, an antigen, an enzyme, a ligand, a receptor, a hormone, and an industrial enzyme.

50. The method of claim 49, wherein the polypeptide is selected from an antibody or antibody fragment, a plasma protein, a cytokine, and a growth factor.

51. A method for screening or selecting cell clones expressing a desired level of a polypeptide, comprising:

a) providing a plurality of cells each comprising an expression cassette comprising a first polynucleotide encoding the polypeptide, at least one stop codon downstream of the first polynucleotide, and a second polynucleotide encoding a cell membrane anchoring peptide, a reporter peptide or an epitope tag downstream of the stop codon;

b) cultivating the cells under conditions that allow expression of the polypeptide; and

c) selecting at least one cell clone expressing the polypeptide fused to a cell membrane anchoring peptide.

52. The method of claim 51, wherein the cells are cultivated in the absence of an aminoglycoside antibiotic.

53. The method of claim 51 or 52, wherein the cell membrane anchoring peptide is a GPI anchor.

54. A method for producing a polypeptide, comprising cultivating a cell line obtained by the method of any of the preceding claims, wherein the cell line is cultivated in the absence of an aminoglycoside antibiotic to allow expression of the polypeptide, and isolating said polypeptide.

55. The method of claim 54, where the polypeptide is a soluble polypeptide that is secreted into a culture medium, and the polypeptide is isolated from said medium.

56. A kit suitable for performing the method of any of the preceding claims.

57. The kit of claim 56, comprising one or more of: (1) at least one kit component comprising an expression cassette as defined in any of claims 1-47; a cell or expression vector comprising said expression cassette; an aminoglycoside antibiotic; or a composition

comprising at least one such component; (2) instructions for practicing a method as defined in any of claims 1-47, instructions for using any component identified in (1) or any composition of any such component; (3) a container for holding said at least one such component or composition, and (4) packaging materials.